Modeling the Fusion and Severing of Neurofilament Polymers in Cells

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Introduction

Neurofilaments are microscopic protein polymers that are abundant in the cytoplasm of nerve cells. They make up an important component of the cytoskeleton, which functions like an internal scaffold to give cells shape and mechanical strength. Neurofilaments are transported along axons and **accumulate abnormally in many neurodegenerative diseases**, such as Amyotrophic lateral sclerosis, Alzheimer's, and Charcot-Marie-Tooth Disease. Neurofilaments can be very long and we believe that their length influences their transport, but **it is not known how length is regulated**. In cortical neurons, filaments have been observed to come together by fusing end-to-end, and it has also been observed that neurofilaments can break apart into two smaller filaments. It seems logical that **a balance between these two actions could work to regulate length.**

Hypothesis

We propose that a dynamic balance of fusion and severing, which causes filaments to lengthen and shorten respectively, regulates length.

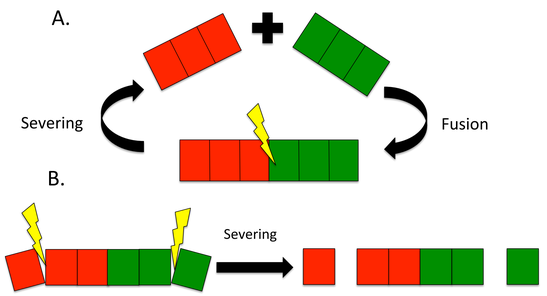
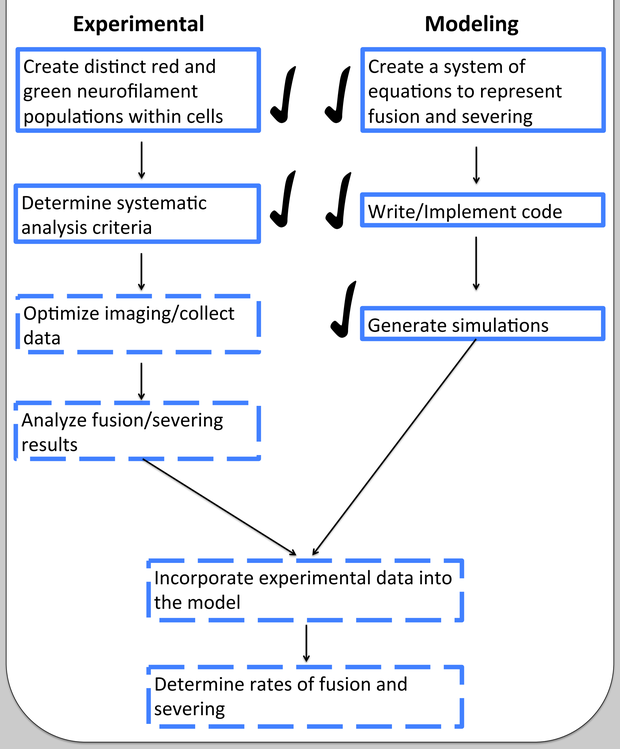


Fig. 1 A. The proposed dynamic balance between end-to-end fusion and severing.

Fig. 2 B. While the appearance of chimeric filaments is explained by end-to-end fusion, the shortening of red and green segments over time is explained by severing, as shown above.

Project Overview



Experimental Strategy

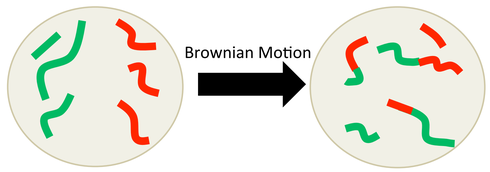


Fig. 2 Illustration of expected neurofilament population if fusion and severing are evident

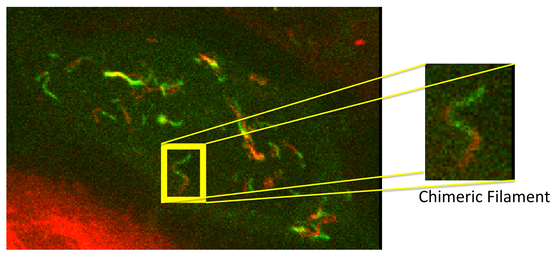


Fig. 3 SW13 vim (-) cells were transfected with fluorescent neurofilament fusion proteins to create distinct populations of red and green neurofilaments as a model system. Chimeric filaments were apparent 9.5 hours after mixing

Experimental Method

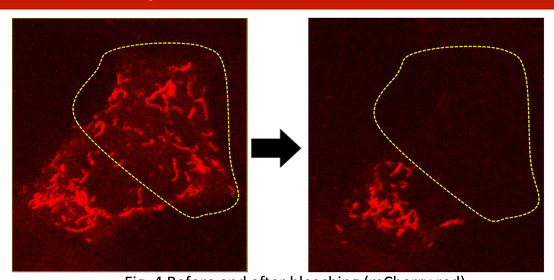


Fig. 4 Before and after bleaching (mCherry red)

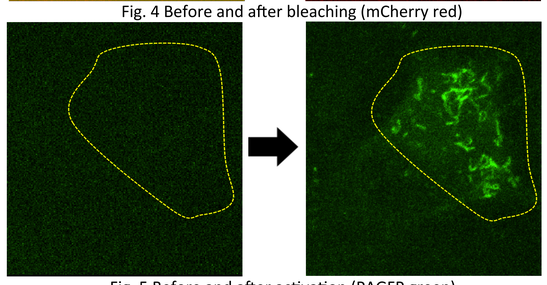


Fig. 5 Before and after activation (PAGFP green)

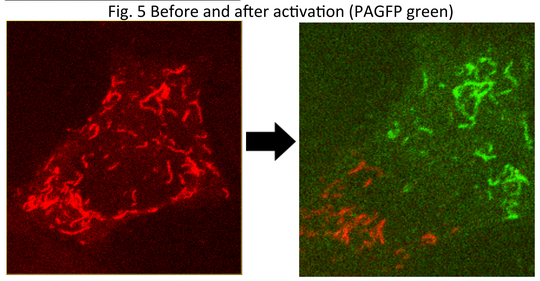


Fig. 6 Before and after bleaching + activation

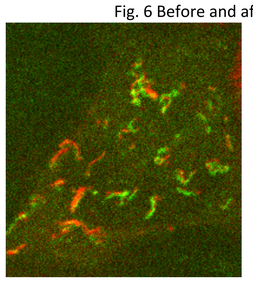


Fig. 7 Neurofilaments after 3.5 hours of mixing

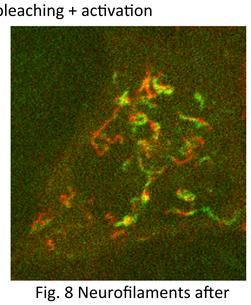


Fig. 8 Neurofilaments after 5.5 hours of mixing

Computational Model

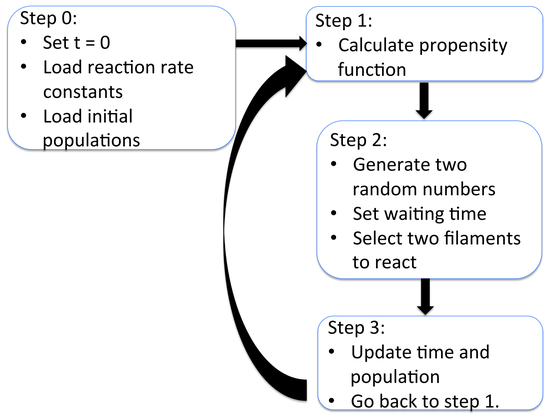
$$F_m + F_n \xrightarrow{A(m,n)} F_{m+n}$$

$$F_{m+n} \xrightarrow{S(m,n)} F_m + F_n$$

1) Color has been considered:

2) Well-mixed system
3) Assume the reaction rates only depend on length (independent of color and configuration)

Stochastic Simulation Algorithm (Gillespie Method)



Results

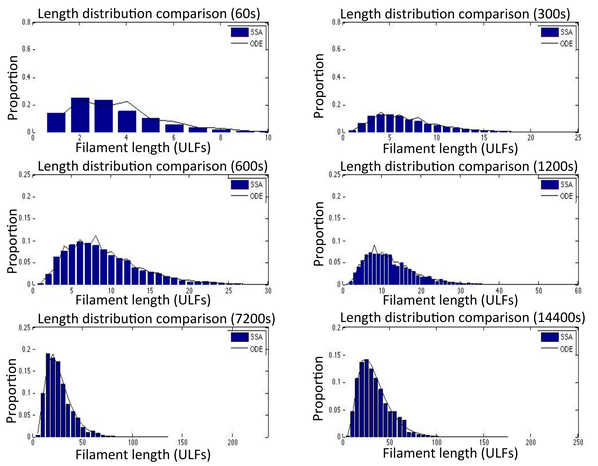


Fig. 9 Length distribution (in ULFs) 60, 300, 600, 1200, 7200 and 14400 sec. The model was initiated with an equal proportion of red and green ULFs. The SSA and Ordinary Differential Equations results are compared (average of five simulations with different random number seeds) and match.

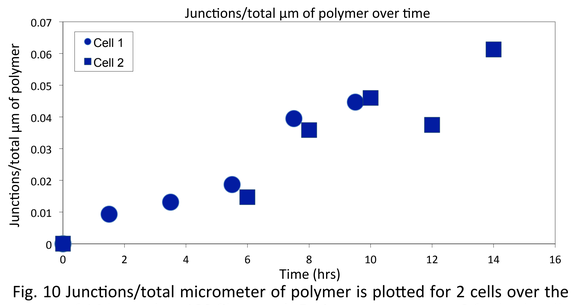


Fig. 10 Junctions/total micrometer of polymer is plotted for 2 cells over the course of 14 hours (n = 100 filaments). Fusion events start at 0 for both cells (due to the filament populations being separated) and continue to occur over the duration of the experiment

Results

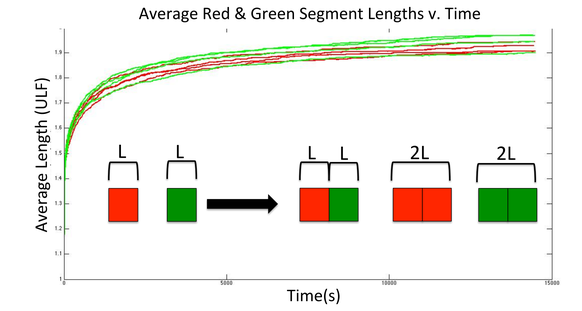


Fig. 11 Average length of red and green segments over time (generated by the SSA model after six iterations with different random number seeds) The model was initiated with an equal proportion of red and green ULFs. The average segment lengths of both colors converge to 2 ULFs with this initial condition.

Fig. 12 Red-Green junctions over time (generated by SSA model after five iterations using different random number seeds). The model was initiated with an equal proportion of red and green ULFs. The rapid increase in junctions over the first 100 seconds is due to the higher probability of shorter filaments reacting.

Conclusions/Future Work

Neurofilament end-to-end fusion occurs and results in the lengthening of these polymers. Preliminary data suggests that fusion is a time-dependent process. SSA modeling has proved to be a viable method to mathematically model neurofilament fusion.

Challenge: Overcome lack of equal expression from fluorescent fusion tags

Solution: Use plasmid bidirectional promoter construct

Next steps:

1) Finish experimental data collection

3) If evidence supports severing, create and incorporate it into the model

4) Implement experimental data into the model

5) Optimize the model to determine fusion and severing rates

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